

CLAIMS

What is claimed is:

1. A method for amplifying a polynucleotide sequence complementary to a target polynucleotide sequence comprising:

(a) hybridizing a polynucleotide comprising a termination polynucleotide sequence to a DNA template-composite primer complex, wherein said complex comprises a composite primer hybridized to a single stranded DNA template comprising the target sequence, said composite primer comprising an RNA portion and a 3' DNA portion,

whereby said polynucleotide comprising a termination polynucleotide sequence is hybridized to a region of the template which is 5' with respect to hybridization of the composite primer to the template;

(b) extending the composite primer in the DNA template-composite primer complex of step (a) with DNA polymerase;

(c) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement,

whereby multiple copies of the complementary sequence of the target sequence are produced.

2. A method for amplifying a polynucleotide sequence complementary to a target polynucleotide sequence comprising:

(a) extending a composite primer in a complex comprising (i) a single stranded DNA template comprising the target sequence; and (ii) the composite primer, said composite primer comprising an RNA portion and a 3' DNA portion, wherein the DNA template is hybridized to the composite primer;

(b) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement,

whereby multiple copies of the complementary sequence of the target sequence are produced.

3. A method for amplifying a polynucleotide sequence complementary to a target polynucleotide sequence comprising:

(a) extending a composite primer in a complex comprising (i) a single stranded DNA template comprising the target sequence; (ii) the composite primer, said composite primer comprising an RNA portion and a 3' DNA portion, wherein the composite primer is hybridized to the DNA template; and (iii) a polynucleotide comprising a termination polynucleotide sequence, wherein the polynucleotide comprising a termination polynucleotide sequence is hybridized to a region of the template which is 5' with respect to hybridization of the composite primer to the template;

(b) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement,

whereby multiple copies of the complementary sequence of the target sequence are produced.

4. A method for amplifying a polynucleotide sequence complementary to a target polynucleotide sequence comprising:

cleaving an RNA portion of a composite primer extension product in a complex comprising (a) a single stranded DNA template comprising the target

sequence; and (b) the composite primer extension product, wherein the composite primer of the composite primer extension product comprises an RNA portion and a 3' DNA portion, wherein the composite primer extension product is hybridized to the DNA template;

wherein said cleaving is with an enzyme that cleaves RNA from an RNA/DNA hybrid, whereby another composite primer hybridizes to the target polynucleotide sequence and repeats primer extension by strand displacement,

whereby multiple copies of the complementary sequence of the target sequence are produced.

5. A method for amplifying a target polynucleotide sequence comprising generating displaced primer extension product using the method of any of claims 1-4, further comprising:

hybridizing a polynucleotide comprising a propromoter and a region which hybridizes to the displaced primer extension product under conditions which allow transcription to occur by RNA polymerase, such that RNA transcripts are produced comprising sequences complementary to the displaced primer extension products,

whereby multiple copies of the target sequence are produced.

6. A method for amplifying a target polynucleotide sequence comprising:

hybridizing a primer extension product with a polynucleotide comprising a propromoter and a region which hybridizes to the primer extension product under conditions which allow transcription to occur by RNA polymerase, such that RNA transcripts are produced comprising sequences complementary to the primer extension product, wherein the primer extension product is a displaced primer extension product generated by:

(a) hybridizing a single stranded DNA template comprising the target sequence with a composite primer, said composite primer comprising an RNA portion and a 3' DNA portion;

(b) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the template which is 5' with respect to hybridization of the composite primer to the template;

(c) extending the composite primer with DNA polymerase;

(d) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement to produce displaced primer extension product;

whereby multiple copies of the target sequence are produced.

7. A method for amplifying a target polynucleotide sequence comprising:

(a) hybridizing a first composite primer to a single stranded DNA template comprising the complementary sequence of the target polynucleotide sequence, said composite primer comprising an RNA portion and a 3' DNA portion, wherein said single stranded DNA template is generated by a method comprising:

(i) hybridizing a polynucleotide comprising the target polynucleotide sequence with a second composite primer, said second composite primer comprising an RNA portion and a 3' DNA portion;

(ii) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the polynucleotide comprising the

target polynucleotide sequence which is 5' with respect to hybridization of the second composite primer to said polynucleotide;

(iii) extending the second composite primer with DNA polymerase;

(iv) cleaving the RNA portion of the annealed second composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the polynucleotide comprising the target polynucleotide sequence and repeats primer extension by strand displacement, whereby multiple copies of a single stranded DNA template comprising the complementary sequence of the target polynucleotide sequence are generated;

(b) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the template which is 5' with respect to hybridization of the first composite primer to the template;

(c) extending the first composite primer with DNA polymerase;

(d) cleaving the RNA portion of the annealed first composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement,

whereby multiple copies of the target polynucleotide sequence are produced.

8. The method of any of claims 1-4 and 6, wherein the RNA portion of the composite primer is 5' with respect to the 3' DNA portion.

9. The method of claim 5, wherein the RNA portion of the composite primer is 5' with respect to the 3' DNA portion.

10. The method of claim 7, wherein the RNA portion of the first composite primer and the second composite primer is 5' with respect to the 3' DNA portion.

11. The method of claim 8, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

12. The method of claim 9, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

13. The method of claim 10, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

14. The method of any of claims 1-4 and 6, wherein the polynucleotide comprising a termination polynucleotide sequence is a template switch oligonucleotide (TSO).

15. The method of claim 5, wherein the polynucleotide comprising a termination polynucleotide sequence is a template switch oligonucleotide (TSO).

16. The method of claim 7, wherein the polynucleotide comprising a termination polynucleotide sequence is a template switch oligonucleotide (TSO).

17. The method of any of claims 1-4 and 6, wherein the polynucleotide comprising a termination polynucleotide sequence is a blocking sequence.

18. The method of claim 5, wherein the polynucleotide comprising a termination polynucleotide sequence is a blocking sequence.

19. The method of claim 7, wherein the polynucleotide comprising a termination polynucleotide sequence is a blocking sequence.

20. The method of claim 5, wherein the termination polynucleotide sequence does not effect template switch under conditions wherein the termination polynucleotide sequence is hybridizable to the DNA template, and wherein the polynucleotide comprising the termination polynucleotide sequence further comprises (a) a propromoter sequence, wherein the propromoter sequence is not hybridizable to the DNA template under conditions wherein the termination polynucleotide sequence is hybridizable to the DNA template; and (b) a sequence which is hybridizable to a complementary sequence of the target polynucleotide sequence.

21. The method of claim 6, wherein the termination polynucleotide sequence does not effect template switch under conditions wherein the termination polynucleotide sequence is hybridizable to the DNA template, and wherein the polynucleotide comprising the termination polynucleotide sequence further comprises (a) a propromoter sequence, wherein the propromoter sequence is not hybridizable to the DNA template under conditions wherein the termination sequence is hybridizable to the DNA template; and (b) a sequence which is hybridizable to a complementary sequence of the target polynucleotide sequence.

22. The method of any of claims 1-4 and 6, wherein the enzyme that cleaves RNA is RNaseH.

23. The method of claim 5, wherein the enzyme that cleaves RNA is RNaseH.

24. The method of claim 7, wherein the enzyme that cleaves RNA is RNaseH.

25. The method of claim 5, wherein the polynucleotide comprising a propromoter and region which hybridizes to the displaced primer extension product is a template switch oligonucleotide (TSO).

26. The method of claim 7, wherein the polynucleotide comprising a propromoter and region which hybridizes to the displaced primer extension product is a template switch oligonucleotide (TSO).

27. The method of claim 5, wherein the polynucleotide comprising the propromoter comprises a region which hybridizes to the displaced primer extension product, whereby DNA polymerase extension of displaced primer extension product produces a double stranded promoter from which transcription occurs.

28. The method of claim 7, wherein the polynucleotide comprising the propromoter comprises a region which hybridizes to the displaced primer extension product, whereby DNA polymerase extension of displaced primer extension product produces a double stranded promoter from which transcription occurs.

29. The method of claim 27, wherein the polynucleotide comprising the propromoter is a propromoter template oligonucleotide (PTO).

30. The method of claim 28, wherein the polynucleotide comprising the propromoter is a propromoter template oligonucleotide (PTO).

31. The method of claim 5, wherein the polynucleotide comprising the propromoter comprises: (a) a termination polynucleotide sequence that does not effect template switch under conditions wherein the termination polynucleotide sequence is hybridizable to the DNA template; (b) a propromoter sequence, wherein the propromoter sequence is not hybridizable to the DNA template under conditions wherein the termination polynucleotide sequence is hybridizable to the DNA template; and (c) a sequence which is hybridizable to a complementary sequence of the target polynucleotide.

32. The method of claim 6, wherein the polynucleotide comprising the propromoter comprises: (a) a termination polynucleotide sequence that does not effect template switch under conditions wherein the termination polynucleotide sequence is hybridizable to the DNA template; (b) a propromoter sequence, wherein the propromoter sequence is not hybridizable to the DNA template under conditions wherein the termination polynucleotide sequence is hybridizable to the DNA template; and (c) a sequence which is hybridizable to a complementary sequence of the target polynucleotide.

33. A method of sequencing a nucleic acid sequence of interest comprising sequencing a nucleic acid amplification product, wherein said nucleic acid amplification product is generated by a method comprising:

- (i) hybridizing a polynucleotide comprising the complement of the sequence of interest with a composite primer, said composite primer comprising an RNA portion and a 3' DNA portion;
- (ii) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the polynucleotide which is 5' with respect to hybridization of the composite primer to said polynucleotide;
- (iii) extending the composite primer with DNA polymerase;

(iv) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the polynucleotide comprising the complement of the sequence of interest and repeats primer extension by strand displacement, whereby nucleic acid amplification products comprising the sequence of interest are generated.

34. A method of sequencing a nucleic acid sequence of interest comprising sequencing a nucleic acid amplification product, wherein said nucleic acid amplification product is generated by a method comprising:

(i) hybridizing a polynucleotide comprising the sequence of interest with a composite primer, said composite primer comprising an RNA portion and a 3' DNA portion;

(ii) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the polynucleotide which is 5' with respect to hybridization of the composite primer to said polynucleotide;

(iii) extending the composite primer with DNA polymerase;

(iv) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the polynucleotide comprising the sequence of interest and repeats primer extension by strand displacement, whereby nucleic acid amplification products comprising the complement of the sequence of interest are generated.

35. A method of detecting whether a nucleic acid sequence of interest is present in a sample, said method comprising detecting whether the sequence of interest is present in a nucleic acid amplification product, wherein said nucleic acid amplification product is generated by:

- (i) hybridizing a target polynucleotide comprising the complement of the sequence of interest with a composite primer, said composite primer comprising an RNA portion and a 3' DNA portion;
- (ii) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the polynucleotide which is 5' with respect to hybridization of the composite primer to said polynucleotide;
- (iii) extending the composite primer with DNA polymerase;
- (iv) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the target polynucleotide and repeats primer extension by strand displacement, whereby amplification products comprising the sequence of interest are generated.

36. A method of detecting presence of a nucleic acid sequence of interest in a sample, said method comprising detecting presence of the sequence of interest in a nucleic acid amplification product, wherein said nucleic acid amplification product is generated by:

- (i) hybridizing a target polynucleotide comprising the sequence of interest with a composite primer, said composite primer comprising an RNA portion and a 3' DNA portion;
- (ii) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the polynucleotide which is 5' with respect to hybridization of the composite primer to said polynucleotide;
- (iii) extending the composite primer with DNA polymerase;
- (iv) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the target polynucleotide and repeats primer extension by strand

displacement, whereby amplification products comprising the complement of the sequence of interest are generated.

37. The method of claim 35 or 36, wherein said sequence of interest comprises a mutation.

38. The method of 37, wherein said mutation is a single nucleotide polymorphism.

39. The method of claim 35, wherein the sequence of interest is detected by hybridizing said amplification product with a nucleic acid probe that is hybridizable to said nucleic acid sequence of interest.

40. The method of claim 36, wherein the sequence of interest is detected by hybridizing said amplification product with a nucleic acid probe that is hybridizable to the complement of the sequence of interest.

41. The method of claim 39 or 40, wherein said nucleic acid probe comprises DNA.

42. The method of claim 39 or 40, wherein said nucleic acid probe comprises RNA.

43. The method of claim 39 or 40, wherein said nucleic acid probe comprises RNA and DNA.

44. The method of claim 39 or 40, wherein said nucleic acid probe comprises peptide nucleic acid (PNA).

45. The method of claim 39 or 40, wherein said probe is provided as a microarray.

46. The method of claim 45, wherein said microarray comprises the probe immobilized on a substrate fabricated from a material selected from the group consisting of paper, glass, plastic, polypropylene, nylon, polyacrylamide, nitrocellulose, silicon, and optical fiber.

47. The method of claim 35, wherein the sequence of interest is detected by limited primer extension.

48. A method for amplifying a target polynucleotide sequence comprising:

(a) hybridizing a single stranded DNA template comprising the target sequence with a composite primer, said composite primer comprising an RNA portion and a 3' DNA portion;

(b) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the template which is 5' with respect to hybridization of the composite primer to the template;

(c) extending the composite primer with DNA polymerase;

(d) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement to produce displaced primer extension product;

(e) hybridizing a polynucleotide comprising a propromoter and a region which hybridizes to the displaced primer extension product under conditions which allow transcription to occur by RNA polymerase, such that RNA transcripts are produced comprising sequences complementary to the displaced primer extension products,

whereby at least 100 copies of RNA transcripts are produced from each displaced primer extension product.

49. The method of claim 48, wherein between 100 to 1000 copies of RNA transcripts are produced from each displaced primer extension product.